170 BET Proteins Mediate Integration Site Selection of MLV Much Alike LEDGF/p75 in HIV Integration

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Background: A hallmark of retroviral replication is stable integration of the viral genome in the host cell DNA. However, retroviruses do not integrate at random. Mediated by LEDGF/p75, lentiviruses preferentially integrate in the body of active transcription units. Gammaretroviruses, including Moloney Murine Leukemia Virus (MLV), favour transcription start sites and CpG islands. It is generally believed that cellular proteins target viral integration complexes to the chromatin resulting in a integration site preference. The consequences of the integration site preference for latency and oncogenicity are still unclear.

Methodology: By co-immunoprecipitation combined with mass spectrometry we identified cellular proteins differentially interacting with HIV-1 and MLV-1 integrase. Hits were ranked and validated resulting in the identification of bromodomain and extra-terminal (BET) proteins (BRD2, BRD3 and BRD4) as gammaretroviral targeting factors.

Results: We discovered that the BET proteins interact with MLV IN and direct integration towards transcription start regions. BET proteins specifically bind and co-localize with the MLV IN in the nucleus of the cell. The interaction is gammaretroviral-specific and mediated by the C-terminal domain of integrase and the extraterminal (ET) domain of BET. Interfering with chromatin interaction of BET proteins via the specific bromodomain inhibitors JQ1 and I-BET decreased MLV replication and MLV vector transduction 5-10-fold, without affecting HIV vector transduction. Quantitative PCR analysis revealed a block at the integration step. In addition, bromodomain inhibitors did not affect the late steps of viral replication. MLV integration site distribution strongly correlated with the BET protein chromatin binding profile. Finally, expression of an artificial fusion protein that merges the BET integrase binding domain with the chromatin interaction domain of LEDGF/p75, retargeted MLV integration into the body of actively transcribed genes, paralleling the HIV integration pattern.

Conclusions: Our results explain the molecular mechanism of MLV integration site selection. Differential integration site selection between HIV and MLV is based on the use of a specific integrase cofactor: BET proteins for MLV and LEDGF/p75 for HIV. Our results provide possibilities to investigate the effect of integration site specificity on the biology of retroviral replication. In addition, our data suggest methods to engineer gammaretroviral vectors with altered integration site specificity which could be used for carrying out gammaretroviral vector-based gene therapy with an increased safety profile.